Hormones of Energy Metabolism in Critically Ill Foals: Insulin, Glucagon, Leptin, Adiponectin, Ghrelin and Growth Hormone

THESIS

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By

Rosa Jessica Irene Maria Barsnick

Graduate Program in Veterinary Clinical Sciences

The Ohio State University

2010

Master's Examination Committee:

Assistant Professor Ramiro Toribio, Advisor
Professor Catherine Kohn
Assistant Professor Margaret Mudge
Copyright by
Rosa Jessica Irene Maria Barsnick
2010
Endocrine dysregulation of energy metabolism is well documented in critically ill humans, but limited information exists in septic foals. The purpose of this study was to provide information on energy metabolism hormonal response in critically ill foals, focusing on insulin, glucagon, leptin and adiponectin, as well as ghrelin and growth hormone (GH), and to determine the association of these hormones with survival in septic, sick non-septic and healthy foals.

We hypothesized that concentrations of insulin, glucagon, leptin, ghrelin, GH and triglycerides will be higher, while adiponectin and glucose will be lower in septic foals than healthy and sick non-septic foals. Magnitude of these differences will be associated with severity of disease and non-survival. This study was a prospective multi-center cross-sectional study, and 44 septic, 62 sick non-septic, and 19 healthy foals of <7 days of age were included. Blood samples were collected at admission. Foals with positive blood culture or sepsis score ≥12 were considered septic.

Septic foals had lower glucose and insulin, and higher triglyceride and glucagon concentrations than healthy foals. Glucagon and adiponectin concentrations were not different between septic foals that died (n = 14) or survived (n = 30). Higher insulin and lower leptin concentrations were associated with mortality. Insulin sensitivity assessed by QUICKI (quantitative insulin sensitivity check index) was increased in septic foals.
Septic foals had significantly higher ghrelin concentrations than sick non-septic foals and healthy controls. GH was higher in hospitalized foals (septic and sick non-septic) compared to healthy foals. Both hormones were negatively correlated with glucose and positively correlated with triglycerides. There was no difference in ghrelin and GH concentrations between septic foals that died (n = 14) or survived (n = 30), but higher ghrelin concentrations were associated with higher sepsis scores.

The endocrine energy response to systemic inflammation and negative energy balance in septic foals is characterized by hypoglycemia, hypertriglyceridemia, low insulin and high glucagon concentrations. Energy endocrine response, especially leptin and insulin, differs between septic foals and critically ill humans. Increases in ghrelin and GH also appear to be associated with the energy status in these foals.
Dedicated to my friends in Wisconsin who are like family to me.
Acknowledgments

I want to thank Ramiro Toribio for his mentorship and guidance in all aspects of this research project and what else was required to successfully complete a master’s program.

Special thanks go to Phoebe Smith who always stood in for me when research seemed to overwhelm me. I am endlessly grateful for her mentorship in the clinical setting of my residency.

I want to acknowledge Catherine Kohn’s support and advice and especially her commitment to the graduate program; and I want to thank Margaret Mudge for her expertise and for filling in for Phoebe Smith on the examination committee.

Thanks go out to all of the clinicians and technical staff at Hagyard Equine Medical Center in Lexington, KY, as well as the Galbreath Equine Center for their support of this project. I also found myself lucky to have Holly Brown, Sam Coe, Brandy Marlow and Krista Hernon to help me in the lab and with data retrieval.

This research was funded by the Morris Animal Foundation – thank you for choosing our project.
Vita

since 07/07 Residency and Graduate Teaching and Research Assistant, Veterinary Clinical Sciences, The Ohio State University, Columbus, OH, USA
10/06 - 06/07 Clinical instructor, Equine Clinic, Internal Medicine, Justus-Liebig-University of Giessen, Germany
04/06 - 07/06 Internship, Wisconsin Equine Clinic and Hospital, Oconomowoc, WI, USA
02/06 - 09/06 Associate, Veterinary practice for horses and small animals, C. Schweer, Isingerode, Germany
02/04 - 12/05 Associate, Bayreuth Equine Clinic, Dr. W. Schill, Eckersdorf, Germany
12/01 - 12/03 Doctorate (Dr. med vet.) Department of Animal Nutrition, School of Veterinary Medicine Hanover, Germany
11/00 - 08/02 Associate, Veterinary practice for horses and small animals, Dr. S. Knorr, Goslar, Germany
10/94 - 06/00 School of Veterinary Medicine Hanover, Germany
Publications


Fields of Study

Major Field: Veterinary Clinical Science
Table of Contents

Abstract......................................................................................................................................... ii

Acknowledgments .................................................................................................................. v

Vita ........................................................................................................................................ vi

List of Tables ........................................................................................................................... ix

List of Figures .......................................................................................................................... xi

Chapter 1: Introduction and Literature Review ..................................................................... 1

Chapter 2: Insulin, glucagon, leptin and adiponectin in critically ill foals ......................... 6
    2.1 Materials and Methods .................................................................................................... 6
    2.2 Results .......................................................................................................................... 11
    2.3 Discussion ....................................................................................................................... 15

Chapter 3: Ghrelin and Growth Hormone in Critically Ill Foals ..................................... 28
    3.1 Material and Methods .................................................................................................... 28
    3.2 Results .......................................................................................................................... 31
    3.3 Discussion ....................................................................................................................... 34

References .................................................................................................................................. 4
List of Tables

Table 2.1. Serum glucose, triglyceride, hormone concentrations and QUICKI in neonatal foals at admission (n = 125) .................................................................21
Table 2.2. Correlation ($r_s$) between blood glucose and triglyceride concentrations, QUICKI and hormones (healthy foals only, n = 19) .................................................................21
Table 2.3. Correlation ($r_s$) between the hormones (healthy foals only, n = 19) ..............22
Table 2.4. Correlation ($r_s$) between blood glucose and triglyceride concentrations, QUICKI and hormones (all sick/hospitalized foals, n = 106) ..................................................22
Table 2.5. Correlation ($r_s$) between the hormones (all sick/hospitalized foals, n = 106) ..22
Table 2.6. Correlation ($r_s$) between blood glucose and triglyceride concentrations, QUICKI and hormones (sick non-septic foals, n = 62) .................................................................23
Table 2.7. Correlation ($r_s$) between the hormones (sick non-septic foals, n = 62) ........23
Table 2.8. Correlation ($r_s$) between blood glucose and triglyceride concentrations, QUICKI and hormones (septic foals only, n = 44) .................................................................24
Table 2.9. Correlation ($r_s$) between the hormones (septic foals only, n = 44) ..............24
Table 2.10. Comparison of serum glucose, triglyceride, hormone concentrations and QUICKI between surviving and non-surviving septic neonatal foals at admission.........25
Table 2.11. Correlation ($r_s$) between sepsis score and hormones/laboratory parameters ..25
Table 2.12. Results for the final model of the multivariate logistic regression for risk factors associated with non-survival in neonatal foals. .............................................26
Table 3.1. Ghrelin, GH, serum glucose and triglyceride concentrations in neonatal foals at admission. .........................................................................................................................37

Table 3.2. Correlation (rₚ) between ghrelin, GH, serum glucose and triglyceride concentrations (healthy foals, n = 19) .........................................................................................................................37

Table 3.3. Correlation (rₚ) between ghrelin, GH, serum glucose and triglyceride concentrations (sick non-septic foals, n = 62).........................................................................................................................2

Table 3.4. Correlation (rₚ) between ghrelin, GH, serum glucose and triglyceride concentrations (septic foals, n = 44).........................................................................................................................2

Table 3.5. Comparison of ghrelin GH, serum glucose and triglycerides between surviving and non-surviving septic neonatal foals at admission.........................................................................................2

Table 3.6. Correlation (rₚ) between sepsis score and ghrelin, GH, glucose and triglycerides.................................................................................................................................3
List of Figures

Figure 2.1. Serum insulin and glucose concentrations in septic foals at admission........27
Chapter 1: Introduction and Literature Review

Sepsis is the most common cause of mortality in foals causing a significant impact on the equine industry.¹⁻⁴ Septic foals often present to intensive care units with anorexia and hypoglycemia. Feed intake and energy metabolism are regulated by the endocrine system, but endocrinopathies are a common sequela of sepsis and have become a major focus of research in critically ill foals.⁵⁻⁸ We believe that disturbances of the energy metabolism in septic neonatal foals are due to sepsis-mediated dysregulation of the endocrine system, but limited information on the pathophysiology of hormonal regulation in septic foals is available.

Maturation of the endocrine system and the associated energy metabolism in the equine neonate is delayed and continues in the post-natal period.⁶⁻¹¹ Neonates are highly dependent on glucose intake and the endocrine glucoregulatory mechanisms are not always fully competent at birth; thus, episodes of hypoglycemia are common in critically ill foals.¹⁰⁻¹² For example, newborn and premature foals have a lower insulin response to increased blood glucose concentrations than older foals and foals born at term.⁹⁻¹³ Post-natal maladaptation and illness are common in foals, making them prone to various diseases, including sepsis. Sepsis is the most common cause of mortality in foals, resulting in major economical losses to the equine industry.¹⁻⁴ Septic foals often present to intensive care units with anorexia and hypoglycemia, and rapid intervention with energy-containing fluids is frequently indicated due to their
inability to nurse or intolerance to enteral feeding. A major complication of parenteral nutrition in foals, including administration of dextrose with intravenous fluids, is the development of hyperglycemia (e.g. glucose intolerance) [14;15]. Hyperglycemia has been associated with increased mortality in critically ill humans [16] due to oxidative stress, glucotoxicity and β-cell dysfunction [17].

Endocrinopathies have become a major focus of research in critically ill humans, and more recently in foals [6-8]. Insulin resistance and hyperglycemia are common manifestations of endocrine dysregulation in people with sepsis or endotoxemia [18-21] and tight glycemic control utilizing insulin therapy has been shown to increase survival in these patients [22]. Similarly, insulin is frequently used in equine neonates when hyperglycemia occurs [23] however, limited information on the hormonal control of energy metabolism in septic neonatal foals is available.

Insulin is essential for energy regulation; it increases cellular glucose uptake, glycogenesis, fatty acid synthesis, and potassium cellular uptake, decreases proteinolysis, lipolysis and gluconeogenesis, and is a vasodilator to microcirculation. Insulin also has anti-inflammatory properties by decreasing inflammatory cytokines and enhancing anti-inflammatory mediators [19], which may confer some therapeutic benefit in patients in a pro-inflammatory state (e.g. septicemia/endotoxemia). Insulin resistance is a common feature of sepsis and endotoxemia in humans [22;24] and has also been reported in horses [25;26].

The quantitative insulin sensitivity check index, QUICKI, is a novel index of insulin sensitivity based on a single fasting blood sample [27]. QUICKI provides a reproducible and robust estimate of insulin sensitivity in humans, as it has strong correlations with insulin.
sensitivity indices calculated from standard glucose clamp or FSIVGTT (frequently sampled intravenous glucose tolerance test) studies in humans, and also appears useful in rodents. QUICKI is a reliable index in insulin-resistant subjects. The authors did not find any reference on the use of QUICKI in the equine species.

Glucagon is secreted by the α-cells of the pancreas and has opposing physiologic roles to insulin as it stimulates gluconeogenesis, glycogenolysis and lipolysis.

Hyperglucagonemia has been reported in septic and endotoxemic humans as well as in endotoxemic dogs and septic rats. Glucagon is thought to be important to maintain or enhance gluconeogenesis in the catabolic state of critical illness.

Leptin, an adipocyte-derived hormone (adipokine), is considered the main regulator of satiety and its blood concentrations have been correlated with total body fat in horses, humans, dogs and other species. Leptin concentrations decrease in feed restricted mares, increase following a meal in humans and leptin increases insulin sensitivity in humans. In addition to energy homeostasis, leptin has immunomodulatory properties, its synthesis is stimulated by inflammatory cytokines and leptin has been described as an acute phase reactant. Increased leptin concentrations have been associated with sepsis and endotoxemia in adult humans, rodents and dogs. However, an increase of leptin concentrations was neither shown in septic human neonates, nor in sheep, cattle or pigs during experimental endotoxemia. No information is available on leptin in horses (foals) with septicemia or endotoxemia.

Adiponectin, another adipokine, is negatively correlated with body fat mass in horses and other species, yet also increases insulin sensitivity. Adiponectin promotes the cell
membrane translocation GLUT4, increases glycolysis and fatty acid oxidation. Studies in mice and humans have shown potential anti-inflammatory and LPS-neutralizing properties of adiponectin.

Ghrelin is produced by gastric ghrelin cells, which are oxyntic cells of the stomach that are characterized by round, compact, electron-dense secretory granules of the P/D₁ type in humans, A-like type in the rat and X type in the dog, also referred to as X/A-like cells. Ghrelin acts via the vagus nerve and is mainly orexigenic (increases food intake). Upon food intake ghrelin release is suppressed by glucose, thus serum levels drop after a meal. However, it is still unclear whether glucose directly or insulin causes the inhibition of ghrelin secretion. On the other hand, ghrelin secretion increases with anorexia. Another important function of ghrelin is that it stimulates the secretion of growth hormone from the pituitary gland.

Growth hormone (GH) is a predominantly anabolic hormone that stimulates cell reproduction and growth mediated by Insulin-like growth factors. GH additionally has direct catabolic effects, e.g. enhanced lipolysis in fat cells and anti-insulin activity causing restriction of glucose transport into cells.

Ghrelin is one of the first hormones to rapidly increase in human and canine endotoxemia and sepsis. Likewise, increased GH secretion has been observed in sepsis and endotoxemia in humans and rodents. Ghrelin’s potential to down-regulate proinflammatory cytokines and inhibit NF-κB has been studied in vitro and in vivo. The protective effect of ghrelin in sepsis and endotoxemia has become a major focus of recent research in critically ill humans, especially in regards to treatment with
ghrelin. To the author’s knowledge, no information is available on ghrelin and growth hormone in critically ill foals.

The purpose of this study was to investigate endocrine aspects of energy metabolism in critically ill foals by determining serum/plasma concentrations of insulin, glucagon, leptin and adiponectin, as well as ghrelin and growth hormone, in healthy, sick non-septic and septic foals, as well as their association with serum glucose and triglyceride concentrations, severity of disease (sepsis score) and outcome (death/survival). We hypothesized that blood concentrations of insulin, glucagon, leptin, ghrelin and growth hormone would be higher while adiponectin would be lower in septic foals compared to healthy controls. We also expected an association of the magnitude of these differences with degree of sepsis and non-survival.
Chapter 2: Insulin, glucagon, leptin and adiponectin in critically ill foals

2.1 Materials and Methods

Animals

Foals ≤7 days old of any breed or sex admitted to The Ohio State University Galbreath Equine Center (OSU) and Hagyard Equine Medicine Institute (HEMI) during foaling season of 2008 were included. Hospitalized foals were classified into one of the two groups: sick non-septic and septic foals. Foals in the septic group had a sepsis score of ≥12 and/or a positive blood culture. Foals in the sick non-septic group were hospitalized for illnesses other than sepsis (e.g. meconium impaction, hypoxic ischemic encephalopathy, failure of transfer of passive immunity, flexural deformities) requiring hospitalization. These foals had negative blood cultures and a sepsis score of ≤11. The control group consisted of 18-24 hours old foals examined on a routine basis at breeding farms in Kentucky. Foals included in the control group were born at the farm and were clinically healthy based on physical exam, a normal complete blood count (CBC), biochemistry profile, a serum immunoglobulin G (IgG) concentration >800 mg/dL and a sepsis score of ≤4. Foals with a history of receiving glucose-containing fluids or corticosteroids prior to admission to the hospital were excluded from the study.
Any foal that was discharged from the hospital was defined as a survivor. Foals that died or were euthanized due to a grave medical prognosis were defined as non-survivors. Individuals euthanized for other reasons such as financial constraints were excluded from the study.

This study was approved by the Ohio State University Veterinary Teaching Hospital executive committee, the Institutional Animal Care and Use Committee, and adheres to the principles of humane treatment of animals in veterinary clinical investigations as stated by the American College of Veterinary Internal Medicine and National Institute of Health guidelines.

**Clinical Information**

History obtained upon admission included expected foaling date, duration of pregnancy, parity, maternal illness, premature lactation, observed or assisted parturition, dystocia, passing and appearance of the fetal membranes and medications (mare and foal). Clinical data collected included signalment (sex, gestational and actual age, breed), physical examination findings, CBC, biochemistry profile including serum glucose, fibrinogen, L-lactate, IgG and triglyceride concentrations. For consistency, the sepsis score was calculated by the first author for each foal individually, based on recorded history, physical exam, and laboratory findings. 

71
Sampling

Blood samples for hormone assays from foals admitted to both hospitals were obtained on admission via sterile jugular venous catheterization. Blood was placed in plain serum clot tubes and chilled aprotinin-EDTA tubes. Aprotinin was added to preserve sample integrity by preventing potential protease degradation of hormones (500 kU/mL of whole blood). The samples were stored in ice water and centrifuged within 12 hours at 5 °C, 2,000 g for 12 minutes. Serum and plasma were then aliquoted and stored at -80 °C until analyzed. A 12 hour delay occurred in few samples, most samples were processed within 2 hours, and from human studies, there is no evidence that sample storage significantly affects stability of insulin, leptin, or adiponectin. Human leptin is stable for 1 week at room temperature and months at 4 °C. Human adiponectin is stable for 2 weeks at 2-8 °C and 1 day at room temperature, according to GenWay Biotech, San Diego, CA 92121(Human Adiponectin ELISA). The processing and storage in this study were performed similarly to routine processing of human samples for immunoassays.

Blood samples for CBC, serum biochemistry and IgG were processed immediately by the respective in-house laboratories (HDM and OSU). Samples from healthy control foals were obtained during routine newborn foal examinations at the farm and processed the same day.

(i) Cell-Dyn 3500R analyzer, Abbott Laboratories, Abbott Park, IL
(ii) Boehringer Mannheim/Hitachi 911 system, Boehringer Mannheim Corporation, Indianapolis, IN
Hormone Concentrations

Blood concentrations of insulin (serum), glucagon (plasma), and adiponectin (serum) were determined using human radioimmunoassays \(^{iii,iv,v}\) while leptin (serum) was measured with a multispecies leptin radioimmunoassay. \(^vi\) All assays have previously been validated for the equine species. \(^{33,37,73,74}\)

Quantitative Insulin Sensitivity Check Index (QUICKI)

Insulin sensitivity was assessed using the quantitative insulin-sensitivity check index \(\text{QUICKI}\), calculated by following formula:

\[
\text{QUICKI} = 1/(\log \text{glucose [mg/dl]} + \log \text{insulin [µIU/ml]})^{27}
\]

\(^{iii}\) Coat-A-Count\(^\text{®}\) human insulin radioimmunoassay, Siemens Healthcare Diagnostics Los Angeles, CA

\(^{iv}\) Coat-A-Count\(^\text{®}\) human glucagon radioimmunoassay, Siemens Healthcare Diagnostics, Los Angeles, CA

\(^{v}\) Human adiponectin radioimmunoassay, Linco\(^\text{®}\), Millipore, St. Charles, MO

\(^{vi}\) Multispecies leptin radioimmunoassay, Linco\(^\text{®}\), Millipore, St. Charles, MO
Data Analysis

Shapiro-Wilk statistic was used to assess the data normality. Only glucose and adiponectin concentrations were normally distributed. The remainder of the data was not normally distributed. Therefore, median and interquartile ranges were calculated for continuous variables. Nonparametric comparisons between the groups were computed with the Kruskal-Wallis statistic and a Dunn’s post-test to compare each group individually, using a statistical software program\(^\text{vii}\). The Mann-Whitney-U test was applied to compare survivors with non-survivors. Significance was set at \(P < 0.05\). The Spearman rank order \((r_s)\) was used to define correlations between variables\(^\text{viii}\).

Continuous variables were categorized by cutoff values based on distribution within a group, and analyzed using logistic regression (procgenmod\(^\text{ix}\)) for binomial distribution. Crude odds ratios and 95% confidence intervals were determined based on categories. The dependent variable was survival/non-survival. All variables were screened and any variables with a \(P\) value <0.25 were tested in a forward and backward stepwise multivariate logistic regression to determine a final model. The Hosmer and Lemeshow Goodness-of-Fit was determined using proc logistic.\(^\text{75}\) Variables that resulted in a \(P\) value <0.05 were retained in the model.

---

\(^\text{vii}\) Prism, version 4.0a, GraphPad Software Inc, San Diego, CA

\(^\text{viii}\) SigmaStat 3.5, Systat, Chicago, IL

\(^\text{ix}\) SAS version 9.1, SAS Institute Inc, Cary, NC
2.2 Results

Study Population

A total of 125 neonatal foals were included, of which 106/125 were hospitalized and 19/125 were healthy foals. Forty-four/106 (41%) were classified as septic, 62/106 (58%) as sick non-septic. Of the 44 septic foals, 30 foals (68%) survived to discharge from the hospital and 32 had a positive blood culture (73%). The median age of all hospitalized foals at admission was 12 hours (range: sick non-septic 1-168 hours; septic 1-192 hours). Healthy controls were all between 18 and 24 hours old.

All healthy controls were Thoroughbreds (n=19). Breeds representing the group of hospitalized foals included Thoroughbred (n=71), Quarter Horse (n = 11), Standardbred (9), Appaloosa (4), Warmblood (3), Friesian (2), American Paint Horse (2), Arabian (1), Gypsy Vanner (1), Percheron (1) and 1 mixed breed. Of the hospitalized foals 50 were fillies and 56 were colts, however, 15/19 healthy controls were fillies.

Serum Glucose and Triglycerides

Serum glucose concentrations were significantly lower in septic foals compared to sick non-septic and healthy foals (p<0.001). Septic and sick non-septic foals had significantly higher serum triglyceride concentrations than the healthy controls (p<0.001, table 1). Glucose and triglyceride concentrations were inversely correlated in all sick/hospitalized foals of the study population as well as in septic foals only (tables 3a and 5a), but not in healthy foals.
Insulin, Glucagon, Leptin and Adiponectin Concentrations

Septic foals had significantly lower insulin and higher glucagon concentrations than the healthy foals (p<0.001). Glucagon concentrations were higher and insulin concentrations were lower in sick non-septic than in healthy foals (p<0.05 and p<0.001, respectively). Glucagon concentrations were also higher in septic than in sick non-septic foals (p<0.01). Insulin was not different between septic and sick non-septic foals. There was no difference in leptin and adiponectin concentrations between groups (table 1).

Glucagon was positively correlated with leptin in hospitalized foals and in septic foals only (tables 3b and 5b). Leptin was negatively correlated with adiponectin in hospitalized foals and in sick non-septic foals only (tables 3b and 4b). There were no correlations between individual hormones in healthy foals (table 2b).

Association of Hormone Concentrations with Glucose and Triglycerides

Insulin concentrations were positively correlated with glucose concentrations in all groups. However, glucagon was inversely correlated with glucose only in the healthy controls. When data of all hospitalized foals (sick non-septic and septic) was analyzed, all hormones were correlated with glucose (table 3a), but when sick non-septic foals and septic foals were analyzed as individual groups, there was no association between glucagon, leptin or adiponectin and glucose (tables 4a and 5a). Glucagon was positively correlated with triglycerides in all groups of sick/hospitalized foals (tables 3a, 4a and 5a), but not in healthy foals. Further, there was no correlation between insulin, leptin or adiponectin and triglycerides in any of the groups.
Association of Glucose and Triglyceride Concentrations with Survival

Of all septic foals, 68% survived (30/44). Glucose concentrations were significantly lower in non-survivors than in septic foals that survived. However, we found no difference in triglyceride concentrations between septic non-survivors and septic survivors. Serum triglyceride concentration was one variable that was retained in the final logistic regression model. In the entire foal study population (healthy, sick non-septic and septic foals), multivariate logistic regression showed that overall non-survival was more likely with serum triglyceride concentrations lower than 60 mg/dl (p<0.01).

The eight septic foals with the highest glucose concentrations (147-329 mg/dL) survived. Only two of these eight individuals had a higher insulin response than normo- or hypoglycemic septic foals (figure 1).

Association of Hormone Concentrations with Survival

Among septic foals, we found significantly higher insulin concentrations in non-survivors than in survivors (p<0.001). Septic foals that had insulin concentrations higher than 4 μIU/ml were more likely to die than those who had low insulin on admission (OR 6.0; 95% CI, 1.2–36.4).

Glucagon and adiponectin were not different between the groups. Leptin concentrations were lower in septic foals that died (p<0.05, table 5). Foals with leptin concentrations lower than 1.1 ng/ml were more likely to die (OR 9.8; 95% CI, 1.4–199.5). Likewise, when compared to the whole study population, the likelihood of non-survival was higher in foals with low leptin concentrations (OR 3.98; 95% CI, 1.1-18.7).
Insulin sensitivity (QUICKI)

QUICKI was higher in sick non-septic and septic foals than healthy foals (table 1), but was not different between septic survivors and septic non-survivors (table 6). QUICKI was negatively correlated with leptin in all groups (tables 2a, 3a, 4a, 5a). In all hospitalized foals (sick non-septic and septic), QUICKI was also negatively correlated with insulin concentrations (tables 3a, 4a, 5a). Further, QUICKI was positively correlated with glucagon, adiponectin and triglycerides in septic foals as well as all hospitalized foals (tables 3a and 5a).

Sepsis Score

The sepsis score correlated significantly with insulin, glucagon, triglycerides and QUICKI; however, no correlation was found between the sepsis score and the adipokines (table 7). The sepsis score was the second variable that was retained in the final logistic regression model. In this study population, non-surviving foals had the higher sepsis scores (p<0.01).

Multivariate Analysis

The final model is attached in table 7. The model includes three variables: triglyceride concentrations, cold extremities (yes/no) and sepsis score (<12/≥12). The Hosmer and Lemeshow Goodness-of-Fit test indicates that the data fit the model well (P = 0.98).
2.3 Discussion

In the current study we documented that endocrine response of energy metabolism in critically ill foals is characterized by hypoglycemia, hypertriglyceridemia, low insulin and high glucagon concentrations. Mortality in septic foals was associated with low leptin and high insulin concentrations.

Septic foals had significantly lower blood glucose concentrations than sick non-septic and healthy foals, which was not unexpected, as hypoglycemia is a common finding in critically ill foals.\textsuperscript{4, 15, 71} Among septic foals, non-survivors had lower glucose concentrations than survivors, which is in agreement with a recent study in critically ill foals.\textsuperscript{15} We found hyperglycemia in few critically ill foals of this study, perhaps caused by increases in cortisol and catecholamine concentrations from stress of transportation or illness. Hyperglycemia has also been associated with mortality and normoglycemia with survival in critically ill foals,\textsuperscript{8, 15} but septic foals in our study that were hyperglycemic (glucose >130 mg/dL) survived. The effect of prolonged hyperglycemia was not evaluated in this study.

Serum triglyceride concentrations were elevated in hospitalized foals, further supporting a metabolic response to the increased energy needs. As carbohydrate stores in foals are limited, the high energy demand depletes the carbohydrate stores, leading to mobilization of fat depots. Ultimately, when the liver cannot maintain glucose production from fatty acids, blood triglyceride concentrations increase. Similarly, hypertriglyceridemia is the main feature of altered fat metabolism in critically ill humans.\textsuperscript{24} In calves, glucose concentrations decrease and triglyceride concentrations increase in response to
administration of endotoxin or TNFα. High TNFα concentrations have been measured in septic foals and could have contributed to the hypoglycemia and hypertriglyceridemia found in the septic foals of this study.

We hypothesized that insulin would be higher in critically ill foals, based on what has been observed in adult horses and calves with experimental endotoxemia. Sepsis and endotoxemia induce insulin resistance in humans, horses and mice. However, in our study, insulin was significantly lower in septic and sick non-septic foals as compared to healthy controls, and insulin and glucose were positively correlated in all groups of the study population. This, as well as the inverse relationship between glucose and glucagon indicates an appropriate physiological response of insulin and glucagon to blood glucose concentrations and contrasts the aforementioned studies.

To our knowledge, this is the first study to document the role of glucagon in the endocrine response to sepsis in foals. Higher glucagon concentrations in the septic foals could be interpreted as a physiological response, as glucagon is a catabolic hormone that stimulates gluconeogenesis. Increased glucagon concentrations have also been documented in septic rats and dogs, and endotoxemic humans.

Adiponectin does not appear to be relevant in sick foals, as we did not find differences in adiponectin concentrations between any of the groups. Adiponectin has been studied in healthy horses, but no information is available in pathological conditions of adult horses or foals. Adiponectin increases in human endotoxemia, but decreases in rats with sepsis. Studies in rodents and humans have shown potential anti-inflammatory and LPS-neutralizing properties of adiponectin.
Based on multivariate logistic regression analysis of all foals of the study population, low serum triglyceride concentrations were associated with non-survival, which likely represents a decompensation in fat metabolism in severely affected individuals, in which fat stores may be depleted. The wide range of triglyceride concentrations in non-survivors could have also accounted for the lack of statistical difference in triglyceride concentrations between survivors and non-survivors.

In septic foals of our study insulin concentrations did not exceed those of the healthy controls; however, higher insulin concentrations were associated with increased mortality in the septic foals, suggesting that hyperinsulinemia was a response to systemic inflammation. Insulin dysregulation has been documented in humans with multi-organ dysfunction syndrome secondary to systemic inflammatory response syndrome who developed insulin resistance, hypertriglyceridemia, and finally insulin deficiency from pancreatic β-cell exhaustion.\textsuperscript{24}

Insulin resistance as a response to systemic inflammation has not been evaluated in critically ill foals by glucose clamp or FSIVGTT, and it was not feasible to do in this study. As an alternative to determine insulin sensitivity based on a single sample, we calculated QUICKI. The fact that QUICKI was higher in septic foals compared to the other groups and positively associated with the sepsis score was unanticipated. We were expecting evidence of insulin resistance in septic foals, because insulin resistance is a common feature of sepsis and endotoxemia in humans\textsuperscript{22,24} and has been reported in endotoxemic horses.\textsuperscript{25,26}
Insulin treatment to maintain normoglycemia has been shown to decrease mortality in the human ICU, because hyperglycemia is more detrimental than hypoglycemia. Intensive glucose control was associated with increased mortality as compared to conventional glucose control (glucose target of 81-108 mg/dL versus <180 mg/dL). As tight glucose control remains controversial in human medicine, in foals with hyperglycemia this concept should be based on equine-specific studies rather than extrapolated from human studies. Nevertheless, insulin treatment is frequent practice in critically ill foals, especially when prolonged hyperglycemia develops from parenteral nutrition. In addition, to its glucoregulatory effects, insulin appears to have beneficial anti-inflammatory properties, decreasing pro-inflammatory and increasing anti-inflammatory mediators in humans. In our study, foals were not treated with exogenous insulin, but interestingly, septic foals that had higher endogenous insulin concentrations on admission were more likely to die. This suggests that the protective anti-inflammatory properties of insulin may not apply to endogenous insulin in foals. Non-surviving septic foals had lower leptin concentrations and were more likely to die than those with higher leptin concentrations. This finding is in agreement with a study by Arnalich et al, in which high leptin concentrations in septic human patients were associated with survival. In contrast, leptin concentrations were higher in septic and endotoxemic rodents, dogs and adult humans, as well as in septic children that did not survive. However, other studies found no association between leptin concentrations and septicemia, shock, multiorgan failure or severity of disease in human neonates, children, or endotoxemic sheep and cows. The lower blood glucose and leptin
concentrations in non-surviving septic foals display a physiological response of leptin to increased caloric demand, as has been shown in feed restricted mares. A similar relationship between leptin and glucose has been demonstrated in horses that had an increase in first insulin and then leptin after feeding, indicating that insulin drives an increase in leptin. Conversely, we did not find a correlation between insulin and leptin concentrations. The leptin response to systemic inflammation or sepsis appears to be variable among species, age groups and possibly individuals. Thus it is difficult to make strong conclusions on leptin and its prognostic value in septic foals with the current data, and additional research using foals with various levels of sepsis or a controlled study will be necessary to further address this question.

Although there is evidence that leptin increases insulin sensitivity, leptin concentrations were negatively correlated with QUICKI in all groups. We conclude that the role of leptin in septic foals is too complex to make any conclusions based on these findings. One limitation of this study was the sex bias in the control group (15/19 foals were fillies). It is unclear, how the hormones of energy metabolism are influenced by sex in the neonatal period. In sexual maturity, leptin has been shown to be influenced by sex hormones, however, in the neonatal period it is unlikely that sex hormones play a significant role in hormonal regulation. Another limitation is the use of the sepsis score to define sepsis in this study. Hypoglycemia is one of the variables used to calculate the sepsis score and this has been an accepted approach in the current literature. In regards to hypoglycemia, this may have caused a bias in the analysis. Of the foals defined as septic in this study, 73% had a positive blood culture, so in only 27% of the foals classified as

19
septic by sepsis score only this approach may have confounded the results. Further, QUICKI has not been assessed in horses before. To validate QUICKI in horses, calculated QUICKI should be compared to results of FSIVGTT or hyperinsulinemic euglycemic clamp methods in this species.

We documented numerous expected physiological changes in hormonal regulation of energy metabolism in septic foals. But we also encountered unexpected concentrations of leptin and insulin compared to how they control energy metabolism and hunger in health. We do not believe that insulin has protective properties in foals as described for other species, because higher insulin concentrations were associated with non-survival. Controlled interventional studies are needed to determine the benefit of insulin treatment in neonatal foals. This study shows that extrapolating evidence from the human literature may not be appropriate with regards to insulin in critically ill equine patients. QUICKI could be a valuable tool to assess insulin sensitivity in foals and horses, but additional work would be required. Leptin seems to be involved in the systemic inflammatory response in foal sepsis and could be a potential prognostic indicator of death in septic foals when concentrations are low.
Table 2.1. Serum glucose, triglyceride, hormone concentrations and QUICKI in neonatal foals at admission (n = 125)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy (n = 19)</th>
<th>Sick non-septic (n = 62)</th>
<th>Septic (n = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>145 (110-182)a</td>
<td>133 (34-272)a</td>
<td>87 (3-329)b</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>27 (19-56)a</td>
<td>40 (8-274)b</td>
<td>79 (12-986)b</td>
</tr>
<tr>
<td>Insulin (µIU/ml)</td>
<td>4.9 (3.2-13)a</td>
<td>2.7 (0.04-17)b</td>
<td>2.2 (0.04-16)b</td>
</tr>
<tr>
<td>Glucagon (pg/ml)</td>
<td>86 (24-279)a</td>
<td>170 (5.1-929)b</td>
<td>810 (8.6-1357)c</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>1.4 (0.55-5.6)</td>
<td>1.2 (0.14-6.3)</td>
<td>1.3 (0.20-1.9)</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>700 (517-852)</td>
<td>725 (443-1037)</td>
<td>792 (372-1008)</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.35 (0.30-0.38)a</td>
<td>0.39 (0.27-2.9)a</td>
<td>0.46 (0.23-6.3)b</td>
</tr>
</tbody>
</table>

Values expressed as median and range; different superscripts letter denote statistical differences, p<0.05

Table 2.2. Correlation (r<sub>s</sub>) between blood glucose and triglyceride concentrations, QUICKI and hormones (healthy foals only, n = 19)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Glucose</th>
<th>Triglycerides</th>
<th>QUICKI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r&lt;sub&gt;s&lt;/sub&gt;</td>
<td>P value</td>
<td>r&lt;sub&gt;s&lt;/sub&gt;</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.41</td>
<td>0.039*</td>
<td>-0.32</td>
</tr>
<tr>
<td>Glucagon</td>
<td>-0.52</td>
<td>0.023*</td>
<td>0.12</td>
</tr>
<tr>
<td>Leptin</td>
<td>-0.18</td>
<td>0.462</td>
<td>0.12</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>0.03</td>
<td>0.894</td>
<td>0.23</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.02</td>
<td>0.354</td>
<td>-</td>
</tr>
</tbody>
</table>

*<p<0.05; Insulin was used to calculate QUICKI
Table 2.3. Correlation (rₜ) between the hormones (healthy foals only, n = 19)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Insulin</th>
<th>Glucagon</th>
<th>Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rₛ</td>
<td>P value</td>
<td>rₛ</td>
</tr>
<tr>
<td>Glucagon</td>
<td>-0.33</td>
<td>0.175</td>
<td>-0.33</td>
</tr>
<tr>
<td>Leptin</td>
<td>-0.23</td>
<td>0.338</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*p<0.05

Table 2.4. Correlation (rₛ) between blood glucose and triglyceride concentrations, QUICKI and hormones (all sick/hospitalized foals, n = 106)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Glucose</th>
<th>Triglycerides</th>
<th>QUICKI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rₛ</td>
<td>P value</td>
<td>rₛ</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.33</td>
<td>&lt;0.001*</td>
<td>0.16</td>
</tr>
<tr>
<td>Glucagon</td>
<td>-0.20</td>
<td>0.045*</td>
<td>0.55</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.24</td>
<td>0.01*</td>
<td>0.11</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>-0.27</td>
<td>0.006*</td>
<td>0.15</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.30</td>
<td>0.001*</td>
<td>-</td>
</tr>
</tbody>
</table>

*p<0.05; ‡Insulin was used to calculate QUICKI

Table 2.5. Correlation (rₛ) between the hormones (all sick/hospitalized foals, n = 106)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Insulin</th>
<th>Glucagon</th>
<th>Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rₛ</td>
<td>P value</td>
<td>rₛ</td>
</tr>
<tr>
<td>Glucagon</td>
<td>0.08</td>
<td>0.430</td>
<td>-</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.04</td>
<td>0.673</td>
<td>0.208</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>-0.01</td>
<td>0.937</td>
<td>-0.032</td>
</tr>
</tbody>
</table>

*p<0.05
Table 2.6. Correlation ($r_s$) between blood glucose and triglyceride concentrations, QUICKI and hormones (sick non-septic foals, n = 62)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Glucose</th>
<th></th>
<th>Triglycerides</th>
<th></th>
<th>QUICKI</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_s$</td>
<td>$P$ value</td>
<td>$r_s$</td>
<td>$P$ value</td>
<td>$r_s$</td>
<td>$P$ value</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.34</td>
<td>0.006*</td>
<td>-0.15</td>
<td>0.240</td>
<td>-0.30‖</td>
<td>0.017*‖</td>
</tr>
<tr>
<td>Glucagon</td>
<td>0.03</td>
<td>0.806</td>
<td>0.37</td>
<td>0.002*</td>
<td>-0.10</td>
<td>0.424</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.23</td>
<td>0.067</td>
<td>0.17</td>
<td>0.166</td>
<td>-0.63</td>
<td>0.001*</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>-0.21</td>
<td>0.095</td>
<td>-0.03</td>
<td>0.807</td>
<td>0.36</td>
<td>0.004*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.09</td>
<td>0.483</td>
<td>-</td>
<td>-</td>
<td>-0.13</td>
<td>0.304</td>
</tr>
</tbody>
</table>

* $p<0.05$; ‖ Insulin was used to calculate QUICKI

Table 2.7. Correlation ($r_s$) between the hormones (sick non-septic foals, n = 62)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Insulin</th>
<th></th>
<th>Glucagon</th>
<th></th>
<th>Leptin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_s$</td>
<td>$P$ value</td>
<td>$r_s$</td>
<td>$P$ value</td>
<td>$r_s$</td>
<td>$P$ value</td>
</tr>
<tr>
<td>Glucagon</td>
<td>0.050</td>
<td>0.728</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.14</td>
<td>0.265</td>
<td>0.21</td>
<td>0.096</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>-0.24</td>
<td>0.062</td>
<td>-0.22</td>
<td>0.079</td>
<td>-0.41</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*p<0.05*
Table 2.8. Correlation ($r_s$) between blood glucose and triglyceride concentrations, QUICKI and hormones (septic foals only, n = 44)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Glucose</th>
<th></th>
<th></th>
<th>QUICKI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_s$</td>
<td>$P$ value</td>
<td>$r_s$</td>
<td>$P$ value</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.56</td>
<td>&lt;0.001*</td>
<td>0.23</td>
<td>0.145</td>
</tr>
<tr>
<td>Glucagon</td>
<td>-0.04</td>
<td>0.798</td>
<td>0.56</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.20</td>
<td>0.190</td>
<td>0.02</td>
<td>0.869</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>-0.29</td>
<td>0.064</td>
<td>0.21</td>
<td>0.180</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.40</td>
<td>0.008*</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* $p<0.05$; † Insulin was used to calculate QUICKI

Table 2.9. Correlation ($r_s$) between the hormones (septic foals only, n = 44)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Insulin</th>
<th></th>
<th></th>
<th>Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_s$</td>
<td>$P$ value</td>
<td>$r_s$</td>
<td>$P$ value</td>
</tr>
<tr>
<td>Glucagon</td>
<td>0.25</td>
<td>0.101</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Leptin</td>
<td>-0.12</td>
<td>0.462</td>
<td>0.35</td>
<td>0.021*</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>-0.13</td>
<td>0.397</td>
<td>0.25</td>
<td>0.101</td>
</tr>
</tbody>
</table>

* $p<0.05$
Table 2.10. Comparison of serum glucose, triglyceride, hormone concentrations and QUICKI between surviving and non-surviving septic neonatal foals at admission

<table>
<thead>
<tr>
<th>Variable</th>
<th>Survivors (n = 30)</th>
<th>Non-survivors (n = 14)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>109 (24-329)</td>
<td>40 (3.0-138)</td>
<td>0.013*</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>159 (12-986)</td>
<td>75 (15-588)</td>
<td>0.780</td>
</tr>
<tr>
<td>Insulin (µIU/ml)</td>
<td>1.6 (0.04-13.3)</td>
<td>3.9 (0.04-15.6)</td>
<td>0.048*</td>
</tr>
<tr>
<td>Glucagon (pg/ml)</td>
<td>566 (23-1228)</td>
<td>516 (8.6-1357)</td>
<td>0.749</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>1.3 (0.3-1.8)</td>
<td>1.0 (0.2-1.7)</td>
<td>0.023*</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>770 (372-1008)</td>
<td>800 (660-985)</td>
<td>0.457</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.44 (0.23-6.3)</td>
<td>0.50 (0.36-2.9)</td>
<td>0.597</td>
</tr>
</tbody>
</table>

Values expressed as median and range, *p<0.05

Table 2.11. Correlation (rₛ) between sepsis score and hormones/laboratory parameters

<table>
<thead>
<tr>
<th>Sepsis Score</th>
<th>rₛ</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>-0.18</td>
<td>0.031*</td>
</tr>
<tr>
<td>Glucagon</td>
<td>0.35</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Leptin</td>
<td>-0.15</td>
<td>0.123</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>0.17</td>
<td>0.077</td>
</tr>
<tr>
<td>Glucose</td>
<td>-0.47</td>
<td>&lt;0.0001*I</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.46</td>
<td>0.0002*</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.04</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*p<0.05; IGlucose was used to calculate sepsis score
Table 2.12. Results for the final model of the multivariate logistic regression for risk factors associated with non-survival in neonatal foals.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides (mg/dl)</td>
<td></td>
<td></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>&lt;30</td>
<td>98.4</td>
<td>7.0-3585.3</td>
<td></td>
</tr>
<tr>
<td>31-60</td>
<td>45.8</td>
<td>2.0-2205.2</td>
<td></td>
</tr>
<tr>
<td>&gt;61</td>
<td>Reference</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Cold extremities</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>no</td>
<td>0.02</td>
<td>0.02-0.22</td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>Reference</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Sepsis score</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>&lt;12</td>
<td>0.04</td>
<td>0.03-0.54</td>
<td></td>
</tr>
<tr>
<td>≥12</td>
<td>Reference</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

OR = Odds ratio, 95% CI = 95% Confidence interval, Reference = Reference group for comparison, N/A = Not applicable
Figure 2.1. Serum insulin and glucose concentrations in 30 septic surviving (△) and 14 septic non-surviving (▲) neonatal foals at admission. The eight septic foals with the highest glucose concentrations (147-329 mg/dL, on right to the dashed line) were survivors.
Chapter 3: Ghrelin and Growth Hormone in Critically Ill Foals

3.1 Material and Methods

Animals

Foals ≤7 days old of any breed or sex admitted to The Ohio State University Galbreath Equine Center (OSU) and Hagyard Equine Medicine Institute (HEMI) during foaling season of 2008 were included. Hospitalized foals were classified into one of the two groups: sick non-septic and septic foals. Foals in the septic group had a sepsis score of ≥12 and/or a positive blood culture. Foals in the sick non-septic group were hospitalized for illnesses other than sepsis (e.g. meconium impaction, hypoxic ischemic encephalopathy, failure of transfer of passive immunity, flexural deformities) requiring hospitalization. These foals had negative blood cultures and a sepsis score of ≤11. The control group consisted of 18-24 hours old foals examined on a routine basis at breeding farms in Kentucky. Foals included in the control group were born at the farm and were clinically healthy based on physical exam, a normal complete blood count (CBC), biochemistry profile, a serum immunoglobulin G (IgG) concentration >800 mg/dL and a sepsis score of ≤4. Foals with a history of receiving glucose-containing fluids or corticosteroids prior to admission to the hospital were excluded from the study.

Any foal that was discharged from the hospital was defined as a survivor. Foals that died or were euthanized due to a grave medical prognosis were defined as non-survivors.
Individuals euthanized for other reasons such as financial constraints were excluded from the study.

This study was approved by the Ohio State University Veterinary Teaching Hospital executive committee, the Institutional Animal Care and Use Committee, and adheres to the principles of humane treatment of animals in veterinary clinical investigations as stated by the American College of Veterinary Internal Medicine and National Institute of Health guidelines.

Clinical Information

History obtained upon admission included expected foaling date, duration of pregnancy, parity, maternal illness, premature lactation, observed or assisted parturition, dystocia, passing and appearance of the fetal membranes and medications (mare and foal). Clinical data collected included signalment (sex, gestational and actual age, breed), physical examination findings, CBC, biochemistry profile including serum glucose, fibrinogen, L-lactate, IgG and triglyceride concentrations. For consistency, the sepsis score was calculated by the first author for each foal individually, based on recorded history, physical exam, and laboratory findings.  

Sampling

Blood samples for hormone assays from foals admitted to both hospitals were obtained on admission via sterile jugular venous catheterization. Blood was placed in plain serum clot tubes and chilled aprotinin-EDTA tubes. Aprotinin was added to preserve sample
integrity by preventing potential protease degradation of hormones (500 kU/mL of whole blood). The samples were stored in ice water and centrifuged within few hours at 5 °C, 2,000 g for 12 minutes. Serum and plasma were then aliquoted and stored at -80 °C until analyzed. Blood samples for CBC\textsuperscript{x}, serum biochemistry and IgG\textsuperscript{xi} were processed immediately. Samples from healthy control foals were obtained during routine newborn foal examinations at the farm and processed the same day.

**Hormone Concentrations**

Plasma ghrelin and GH concentrations were determined using an active ghrelin radioimmunoassay\textsuperscript{xii} and a porcine/canine growth hormone radioimmunoassay\textsuperscript{xiii}. Both assays have previously been validated for the equine species.\textsuperscript{84,85}

**Data Analysis**

Shapiro-Wilk statistic was used to assess the data normality. The data was not normally distributed. Therefore, median and interquartile ranges were calculated for continuous variables. Nonparametric comparisons between the groups were computed with the Kruskal-Wallis statistic and a Dunn’s post-test to compare each group individually, using

\textsuperscript{x} Cell-Dyn 3500R analyzer, Abbott Laboratories, Abbott Park, IL

\textsuperscript{xi} Boehringer Mannheim/Hitachi 911 system, Boehringer Mannheim Corporation, Indianapolis, IN

\textsuperscript{xii} Ghrelin (active) radioimmunoassay, Linco®, Millipore, St. Charles, MO

\textsuperscript{xiii} Porcine/canine growth hormone radioimmunoassay, Linco®, Millipore, St. Charles, MO
a statistical software program. The Mann-Whitney-U test was applied to compare survivors with non-survivors. Significance was set at \( P < 0.05 \). The Spearman rank order \( (r_s) \) was used to define correlations between variables. Continuous variables were categorized by cutoff values based on distribution within a group, and analyzed using logistic regression (procgenmod) for binomial distribution. Crude odds ratios and 95% confidence intervals were determined based on categories. The dependent variable was survival/non-survival. All variables were screened and any variables with a \( P \) value \(<0.25 \) were tested in a forward and backward stepwise multivariate logistic regression to determine a final model. The Hosmer and Lemeshow Goodness-of-Fit was determined using proc logistic. Variables that resulted in a \( P \) value \(<0.05 \) were retained in the model.

### 3.2 Results

**Study Population**

A total of 125 neonatal foals were included, of which 106/125 were hospitalized and 19/125 were healthy foals. Forty-four/106 (41%) were classified as septic, 62/106 (58%) as sick non-septic. Of the 44 septic foals, 30 foals (68%) survived to discharge from the hospital and 32 had a positive blood culture (73%). The median age of all hospitalized foals

---

\(^{xiv}\) Prism, version 4.0a, GraphPad Software Inc, San Diego, CA

\(^{xv}\) SigmaStat 3.5, Systat, Chicago, IL

\(^{xvi}\) SAS version 9.1, SAS Institute Inc, Cary, NC
foals at admission was 12 hours (range: sick non-septic 1-168 hours; septic 1-192 hours). Healthy controls were all between 18 and 24 hours old.

All healthy controls were Thoroughbreds (n=19). Breeds representing the group of hospitalized foals included Thoroughbred (n=71), Quarter Horse (n = 11), Standardbred (9), Appaloosa (4), Warmblood (3), Friesian (2), American Paint Horse (2), Arabian (1), Gypsy Vanner (1), Percheron (1) and 1 mixed breed. Of the hospitalized foals 50 were fillies and 56 were colts, however, 15/19 healthy controls were fillies.

Serum Glucose and Triglycerides

Serum glucose concentrations were significantly lower in septic foals compared to sick non-septic and healthy foals (p<0.001). Septic and sick non-septic foals had significantly higher serum triglyceride concentrations than the healthy controls (p<0.001, table 1). Glucose and triglyceride concentrations were inversely correlated in septic foals (table 4), but not in healthy foals (table 2).

Association of Glucose and Triglyceride Concentrations with Survival

Of all septic foals, 68% survived (30/44). Glucose concentrations were significantly lower in non-survivors than in septic foals that survived. However, we found no difference in triglyceride concentrations between septic non-survivors and septic survivors. Serum triglyceride concentration was one variable that was retained in the final logistic regression model. In the entire foal study population (healthy, sick non-septic and
septic foals), multivariate logistic regression showed that overall non-survival was more likely with serum triglyceride concentrations lower than 60 mg/dl (p<0.01).

**Ghrelin and Growth Hormone Concentrations**

Septic foals had significantly higher ghrelin concentrations than sick non-septic foals and healthy controls (p<0.001). GH concentrations were higher in septic foals and sick non-septic foals compared to healthy foals (p<0.05, table 1).

**Association of Hormone Concentrations with Glucose and Triglycerides**

Ghrelin and GH were negatively correlated with glucose in healthy foals (table 2). In septic foals, ghrelin was positively correlated with triglycerides, and GH was negatively correlated with glucose (table 4). Ghrelin and GH were not correlated in any group.

**Association of Hormone Concentrations with Survival**

There was no statistical difference in ghrelin or growth hormone concentrations between septic survivors and non-survivors (table 5). However, logistic regression showed that foals with ghrelin concentrations lower than 15 pg/ml were less likely to die (OR 0.23; 95% CI, 0.05-0.79) than foals with concentrations higher than 15 pg/ml. Likewise, the likelihood of non-survival was lower in foals with GH concentrations less than 1.8ng/ml (OR 0.21; 95% CI, 0.05-0.73).
Sepsis Score

The sepsis score correlated significantly with ghrelin and triglyceride concentrations (positive correlation), as well as glucose (negative correlation). No correlation was found between the sepsis score and GH (table 6). The sepsis score was the second variable that was retained in the final logistic regression model. In this study population, non-surviving foals had the higher sepsis scores (p<0.01).

Multivariate Analysis

The final model is described in table 7. The model includes three variables: triglyceride concentrations, cold extremities (yes/no) and sepsis score (<12/≥12). The Hosmer and Lemeshow Goodness-of-Fit test indicates that the data fit the model well (P = 0.98).

3.3 Discussion

In the current study we documented that the endocrine response of energy metabolism in critically ill foals is characterized by hypoglycemia, hypertriglyceridemia, and an increase in ghrelin and GH concentrations. Increases in ghrelin and GH were associated with decreased serum glucose concentrations and increased serum triglyceride concentrations.

Ghrelin secretion physiologically increases when blood glucose concentrations are low, so the observed association between glucose and ghrelin appears like an appropriate physiological response. The higher ghrelin concentrations in septic foals and the correlation with the sepsis score are in agreement with findings in human abdominal
Ghrelin levels also increased in dogs after administration of endotoxin, but studies in rats remain contradictory. Negative energy balance and anorexia provide an explanation for the increase in plasma ghrelin observed in septic foals. Reduced ghrelin clearance due to hepatorenal injury in sepsis and endotoxemia could be another reason for the observed increase of ghrelin. Ghrelin has recently been called “a signal of insufficient energy intake” and obviously plays a significant role in negative energy balance. In critically ill foals negative energy balance develops from anorexia in conjunction with the higher energy demand of illness, often reflected as hypoglycemia. In humans, ghrelin induces lipolysis in adipocytes. Assuming that the increase in plasma ghrelin, likely stimulated by hypoglycemia similarly promotes lipolysis in horses, ghrelin may be pivotal in the observed increase in serum triglycerides in septic foals.

In mice, blood triglycerides promoted the transport of intravenously administered ghrelin across the blood-brain-barrier, and fasting also tended to promote ghrelin transport across the blood-brain-barrier. Possibly, endogenous ghrelin has a similar effect as exogenous ghrelin, and not only hypoglycemia results in increased ghrelin secretion, but subsequently hypertriglycerideremia contributes to the central signaling of ghrelin to induce hunger and stimulate feed intake. Nevertheless, in critically ill neonatal foals, these proposed mechanisms apparently are not able to overcome anorexia, and other mechanisms and mediators of anorexia still predominate.

Ghrelin is not only a GH secretagogue and orexigenic factor, ghrelin additionally has significant anti-inflammatory properties and is being studied as a treatment for sepsis and
endotoxemia in rodent models. Ghrelin down-regulates proinflammatory cytokines and inhibits NF-κB in vitro\textsuperscript{66} and in vivo\textsuperscript{67,68}. To the author’s knowledge, the anti-inflammatory properties of ghrelin have not been studied in septic or endotoxemic domestic mammals other than rodents. Further research is needed to determine the possible beneficial effects of exogenous ghrelin in critically ill foals.

The increased GH concentration in septic foals was possibly due to the effect of increased ghrelin, although there was no significant correlation between ghrelin and GH. This finding was unexpected, because ghrelin stimulates GH secretion. This leads to the assumption that GH secretion is regulated by other mediators aside from ghrelin. The association between GH secretion and sepsis or endotoxemia has been studied in several species. Recombinant bovine TNF has been shown to mediate GH secretion in dairy heifers,\textsuperscript{94} and GH secretion is stimulated in human sepsis due to an abnormal pituitary response.\textsuperscript{64}

Foals with low GH concentrations were overall less likely to die, but the lack of statistical difference between GH concentrations of septic survivors and non-survivors questions the usefulness of GH as a predictor for mortality in critically ill foals. This is in contrast to studies in septic children\textsuperscript{65} and adult critically ill patients.\textsuperscript{63}

Hypoglycemia and hypertriglyceridemia are common in septic foals. Increases in ghrelin and GH appear to be linked to the energy status in these foals. The observed increases in ghrelin and GH in critically ill foals is in agreement with results of studies in other species\textsuperscript{95} and humans, however, ghrelin and GH are not useful for prediction of mortality.
in septic foals. Possible implications for treatment of septic foals with ghrelin should be further investigated.

Table 3.1. Ghrelin, GH, serum glucose and triglyceride concentrations in neonatal foals at admission.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy (n = 19)</th>
<th>Sick Non-Septic (n = 62)</th>
<th>Septic (n = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghrelin (pg/ml)</td>
<td>7.8 (0.15-21)a</td>
<td>8.9 (0.15-190)a</td>
<td>20 (0.15-779)b</td>
</tr>
<tr>
<td>GH (ng/ml)</td>
<td>1.6 (0.05-4.2)a</td>
<td>2.8 (0.05-35)b</td>
<td>4.9 (0.05-93)b</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>145 (110-182)a</td>
<td>133 (34-272)a</td>
<td>87 (3-329)b</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>27 (19-56)a</td>
<td>40 (8-274)b</td>
<td>79 (12-986)b</td>
</tr>
</tbody>
</table>

Values expressed as median and range, different letter superscripts denote statistical difference, p<0.05

Table 3.2. Correlation ($r_s$) between ghrelin, GH, serum glucose and triglyceride concentrations (healthy foals, n = 19)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Glucose</th>
<th>Triglycerides</th>
<th>Growth Hormone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_s$</td>
<td>$P$ value</td>
<td>$r_s$</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>-0.51</td>
<td>0.025*</td>
<td>0.11</td>
</tr>
<tr>
<td>GH</td>
<td>-0.49</td>
<td>0.034*</td>
<td>-0.03</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.02</td>
<td>0.354</td>
<td>-</td>
</tr>
</tbody>
</table>

* $p<0.05$
Table 3.3. Correlation ($r_s$) between ghrelin, GH, serum glucose and triglyceride concentrations (sick non-septic foals, n = 62)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Glucose</th>
<th>Triglycerides</th>
<th>Growth Hormone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_s$</td>
<td>$P$ value</td>
<td>$r_s$</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>-0.13</td>
<td>0.314</td>
<td>0.004</td>
</tr>
<tr>
<td>GH</td>
<td>0.10</td>
<td>0.437</td>
<td>0.15</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.09</td>
<td>0.483</td>
<td>-</td>
</tr>
</tbody>
</table>

*p<0.05

Table 3.4. Correlation ($r_s$) between ghrelin, GH, serum glucose and triglyceride concentrations (septic foals, n = 44)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Glucose</th>
<th>Triglycerides</th>
<th>Growth Hormone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_s$</td>
<td>$P$ value</td>
<td>$r_s$</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>-0.14</td>
<td>0.381</td>
<td>0.30</td>
</tr>
<tr>
<td>GH</td>
<td>-0.39</td>
<td>0.009*</td>
<td>0.20</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.40</td>
<td>0.008*</td>
<td>-</td>
</tr>
</tbody>
</table>

*p<0.05

Table 3.5. Comparison of ghrelin GH, serum glucose and triglycerides between surviving and non-surviving septic neonatal foals at admission

<table>
<thead>
<tr>
<th>Variable</th>
<th>Survivors (n = 30)</th>
<th>Non-survivors (n = 14)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghrelin (pg/ml)</td>
<td>19.2 (0.15-779.2)</td>
<td>23.5 (2.13-476.1)</td>
<td>0.59</td>
</tr>
<tr>
<td>GH (ng/ml)</td>
<td>4.39 (0.31-93.0)</td>
<td>9.40 (0.05-91.0)</td>
<td>0.10</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>109 (24-329)</td>
<td>40 (3.0-138)</td>
<td>0.013*</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>159 (12-986)</td>
<td>75 (15-588)</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Values expressed as median and range, *p<0.05
Table 3.6. Correlation ($r_s$) between sepsis score and ghrelin, GH, glucose and triglycerides

<table>
<thead>
<tr>
<th></th>
<th>Sepsis Score</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_s$</td>
<td>$P$ value</td>
<td></td>
</tr>
<tr>
<td>Ghrelin</td>
<td>0.42</td>
<td>&lt;0.0001*</td>
<td></td>
</tr>
<tr>
<td>GH</td>
<td>0.10</td>
<td>0.337</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>-0.47</td>
<td>&lt;0.0001*†</td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.46</td>
<td>0.0002*</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05; †Glucose was used to calculate sepsis score
References


